Isolation and Characterization of Volatile Sulfur-Containing Meat Flavor Components in Model Systems

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Reaction of an aqueous solution of cystine with thiamin, glutamate, and ascorbic acid produces a complex mixture of compounds with an overall flavor resembling that of roasted meat. The reaction was carried out at 120 °C for 0.5 h at pH 5.0 in a closed system. The aroma compounds were isolated by means of the simultaneous steam distillation/solvent extraction method according to Likens–Nickerson. The flavor concentrate was preseparated by liquid chromatography on silica gel using a pentane–diethyl ether gradient and subsequently analyzed by GC and GC/MS. Sulfur components were detected by a selective flame photometric detector. Unknown flavor components were isolated by preparative capillary gas chromatography, and the structures were elucidated on the basis of spectroscopic studies. Various heterocyclic thioethers, disulfides, and hemidithioacetals were identified for the first time in the volatiles of the heated meat flavor model mixture. Formation pathways, sensory properties, and spectroscopic data of newly identified flavor components are discussed. In most cases, identifications were confirmed by organic syntheses. Some of the most important mass spectrometric fragmentation pathways are proposed.

It is well-known from literature that many types of chemical reactions are responsible for meat flavor formation due to numerous different water-soluble flavor precursors that generate volatile components mainly on heating. The chemistry of meat flavor formation has been extensively investigated and reviewed by many authors (van den Ouweland et al., 1978; Mac Leod and Seyyedain-Ardebili, 1981; Mac Leod, 1986).

All chemical compounds occurring in *natural* meat volatiles are listed in a recent review article by Shahidi et al. (1986).

A great number of meat flavor components have been isolated from model systems. Studies of reaction flavors or model systems are extremely helpful in the identification of organoleptically interesting meat flavor components.

The literature dealing with model meat flavor systems is so voluminous that it is impossible to give a complete survey. An extremely comprehensive and excellent review dealing with a variety of chemical reactions in model systems generating "meaty" components has been recently published by Mac Leod (1986). On the basis of this literature background, only some newer references since 1983 should be additionally cited (Golovnya et al., 1983; Stewart, 1985; Tateo et al., 1987; Misharina et al., 1987).

Remarkable progress has been made in meat flavor research over the past 10 years.

Meat flavor is probably the result of a number of volatiles from different chemical classes present in particular quantitative proportions. There is no doubt that sulfur-containing components play an important role in roasted and cooked meat flavors because only trace amounts of these types of compounds need be present to be aroma-effective. The investigation of a series of model meat systems has clearly proved the important role of volatile sulfur-containing heterocyclic components substituted with sulfur in the 3-position.

One of these 3-substituted sulfur compounds, 2-methyl-3-(methylthio)furan, was identified recently in cooked beef aroma (Mac Leod and Ames, 1986) and in a heated yeast extract composition (Ames and Mac Leod, 1985) and is considered a meaty character impact compound.

In this context, it is worth mentioning that in natural meat volatiles mainly 2-substituted derivatives of furan or thiophene have been identified. This does not necessarily mean, however, that there are no 3-substituted sulfur derivatives in natural meat products. It is more likely that these components substituted with sulfur in the 3-position are only present in trace amounts in natural meat volatiles.

In addition to analyzing meat, we decided to investigate a relevant model meat flavor system approximating cooked and/or roasted meat that was prepared by heating an aqueous solution of cystine, thiamin, glutamate, and ascorbic acid. The purpose of the present investigation was to study the formation of volatile sulfur-containing components responsible for interesting meaty flavor notes in this model meat system based on naturally occurring precursors.

EXPERIMENTAL SECTION

Preparation of the Reaction Mixture. A mixture of 100 g of cystine, 100 g of thiamin hydrochloride, 100 g of ascorbic acid, 500 g of monosodium glutamate, and 2 L of distilled water was placed in an autoclave equipped with a stirrer arm. The mixture was at pH 5.0. The reaction mixture was heated to 120 °C for 0.5 hr and allowed to cool to room temperature.

Isolation of Volatiles by Simultaneous Distillation/Extraction. The dark brown reaction mixture was placed in a 4-L round-bottom flask and continuously extracted for 7 h with 200 mL of pentane/diethyl ether (1:1) at atmospheric pressure according to the procedure described by Likens–Nickerson. The pentane/ether extract was dried over anhydrous sodium sulfate, and the organic solvent was removed on a 25 cm × 1 cm Vigreux distillation column. The concentrate was stored under nitrogen.

Preseparation by Adsorption Chromatography. A separation according to the polarity of the components was carried out by liquid–solid chromatography. The total flavor extract

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was preseparated in 20 fractions by medium-pressure liquid chromatography on silica gel with a pentane/ether gradient as mobile phase. To this end, the aroma concentrate was placed on a cooled column (480 mm × 37 mm (i.d.)) filled with 240 g of silica gel (25-40 μm). The elution rate was 10 mL/min. All eluates were dried over anhydrous sodium sulfate. The individual fractions were concentrated to a definite volume of 1 mL on a 25 cm × 1 cm Vigreux column. Further concentration to about 100 μL was slowly performed by a procedure described by Dünges (1979).

Capillary Gas Chromatography (HRGC). Analytical separations were performed on a Varian 3700 GC instrument as well as on a Carlo Erba Type 5360 Mega Series gas chromatograph. The Varian 3700 GC system was modified with a hot split/splitless injector and additionally equipped with a commercially available inlet splitter (Gerstel, Mülheim/Ruhr) in order to install two capillary columns of different polarity. The Carlo Erba 5360 gas chromatograph was fitted with a so-called "glass-cap-cross" inlet splitting system. This system for double-column GC analysis in combination with the cold on-column injection technique has been developed in our laboratory quite recently (Bretschneider and Werkhoff, 1988a,b).

Columns used: (1) polar column A, 60 m × 0.32 mm (i.d.) fused silica capillary column coated with DB-WAX (0.25-μm film thickness); (2) nonpolar column B, 60 m × 0.32 mm (i.d.) fused silica capillary column coated with DB-1 (0.25-μm film thickness).

A helium carrier gas flow rate of 2-3 mL/min and an oven temperature programmed from 60 to 220 °C at 3 °C/min were used. The temperature of the injector (Varian 3700) was 250 °C, and the detector temperatures were 275 °C.

Furthermore, GC samples were investigated on a Carlo Erba Type 4200 gas chromatograph fitted with a normal FID and with a flame photometric detector (FPD) operating in the sulfur mode at 394 nm. Again, the dead-volume free glass-cap-cross was used in order to split the carrier gas flow. By means of these sulfur chromatograms, mass spectral evaluation could be focused on certain compounds in very complex mixtures.

Preparative Capillary Gas Chromatography. A Carlo Erba Type 5360 Mega Series gas chromatograph equipped with a flame ionization detector and a split/splitless injector was used to isolate individual components from the eluent of a capillary column.

A glass-cap-cross effluent splitting system was installed in this GC instrument, and the splitting ratio between the detector and the trap was adjusted to be 1:5 or 1:10.

All preparative GC separations were performed on widebore fused silica capillary columns (30 m × 0.53 mm (i.d.), film thickness between 1.0 and 3.0 μm) combining the advantage of sufficient separation efficiency with relatively high sample capacity. A high sample capacity is especially desirable to reduce the number of injections.

Prior to spectroscopic investigations, the collected samples were reinjected into an analytical capillary GC system in order to determine the purity of the condensed components.

GC-Sniffing. For odor evaluation, a further GC instrument was equipped with an all-glass effluent splitter (glass-cap-cross) with one splitter arm going to an FID and the other splitter arm connected via a fused silica capillary to a sniffing port. The components separated by GC were evaluated by their smell at the sniffing port. GC conditions were similar to those mentioned above.

Gas Chromatography/Mass Spectrometry (GC/MS). The column and operating conditions employed for the gas chromatograph in GC/MS analysis were similar to those described above.

For obtaining the mass spectra (EI) two systems were used:

(1) Finnigan MAT Series 8230 instrument interfaced to a Carlo Erba 5360 Mega Series gas chromatograph (open split coupling interfaced via a flexible transfer line). Operating conditions: temperature of the transfer line, 250 °C; temperature of the ion source, 220 °C; electron energy, 70 eV; cathodic current, 1 mA; accelerating voltage, 3 kV; resolution, 900; scan speed, 1 s/dec.

Table I. Chemical Classes of Volatile Sulfur-Containing Components Identified in a Model Meat Flavor System

class	no.
aliphatic mercaptans	3
heterocyclic mercaptans	7
heterocyclic thioethers	11
heterocyclic disulfides	7
thiophenes	22
thiazoles	6
1,2-dithianes	2
1,2-dithiolanes	1
1,2,4-trithiolanes	2
1,2,4,5-tetrathianes	2
thiaalkanethiols (hemidithioacetals)	2
(thiaheteroaryl)alkanethiols	3
miscellaneous	2

(2) MAT Series 112 S instrument interfaced to a Varian 3700 gas chromatograph (directly coupled, 250 °C). Operating conditions: ion source, 230 °C.

The compounds were identified by comparison of the mass spectra and GC retention indices with reference data from authentic components and with data of our own MS library.

IR and NMR Analysis. Infrared spectra were obtained in CCl_4 on a Perkin-Elmer 983 G Type instrument. ^1H NMR spectra were measured at 200 MHz in $CDCl_3$ or C_6D_{12} on a Varian XL-200 instrument with tetramethylsilane as an internal standard.

Organic Syntheses. *Materials.* 2-Methyl-3-furanthiol was purchased from IFF (Union Beach, NJ). 3-Mercaptopropionic acid and 2-chloropropionic acid were obtained from Aldrich (Steinheim, FRG). 5-Chloropentan-2-one was bought from Merck (Darmstadt, FRG).

2-Methyl-3-thiophenethiol was synthesized according to the literature starting with 2-methylthiophene (Steinkopf, 1934; Gronowitz and Raznikiewicz, 1973; Gronowitz and Håkansson, 1960; Brandsma and Bos, 1969). 2-Methylthiophene was purchased from Biesterfeld & Co. (Hamburg, FRG).

1. Synthesis of 2-Methyl-3-[(2-methyl-3-thienyl)dithio]-furan (5). A solution of 500 g of NaOH (20% aqueous solution) was stirred for 10 min at 10-15 °C, when a mixture of 28.5 g (0.25 mol) of 2-methyl-3-furanthiol and 32.5 g (0.25 mol) of 2-methyl-3-thiophenethiol in methylene chloride (150 mL) was added dropwise. Subsequently, the reaction mixture was kept for 15-20 min at the above temperature.

The mixture was allowed to warm to 20-30 °C, and 31 g (0.275 mol) of hydrogen peroxide (30% aqueous solution) was added within 15 min. After 30 min of further stirring at this temperature, the organic phase was separated, washed with several portions of water, and then dried over anhydrous Na_2SO_4. Finally, the organic solvent was expelled by distillation at ambient pressure, and the residual liquid was distilled at reduced pressure; the fraction boiling at 100-136 °C (1 mbar) was collected. In this way 29 g (47.9%) of a product mixture was obtained and the desired disulfide was finally purified by chromatography on silica gel and/or preparative gas chromatography.

IR (CCl_4): 2918, 2851, 1579, 1512, 1437, 1383, 1224, 1175, 1123, 1087, 939, 888, 854, 708, 651, 618, 605 cm⁻¹. ¹H NMR ($CDCl_3$): 2.02 (3 H, s), 2.30 (3 H, s), 6.38 (1 H, d, J = 1.7 Hz), 6.99 (1 H, d, J = 5.3 Hz), 7.06 (1 H, d, J = 5.3 Hz), 7.30 ppm (1 H, d, J = 1.7 Hz). MS: See Figure 1.

2. Synthesis of Bis(2-methyl-3-thienyl) Disulfide (4). This component was prepared according to the same procedure as described above. 2-Methyl-3-thiophenethiol (32.5 g, 0.25 mol) in 150 mL of methylene chloride was added dropwise to a stirred solution of NaOH (250 g, 20% aqueous solution) at 10-15 °C. The mixture was allowed to warm to ambient temperature, and 16.9 g of hydrogen peroxide (0.15 mol) was added over 15 min. The organic layer, carefully washed to neutrality and worked up as usual, afforded 10.0 g (15.5%) of pure bis(2-methyl-3-thienyl) disulfide [distillation at 100-136 °C (1 mbar)].

IR (CCl_4): 2953, 2917, 2868, 2855, 1430, 1375, 1175, 1146, 1089, 878, 853, 707, 619 cm⁻¹. ¹H NMR ($CDCl_3$): 2.22 (6 H, s), 6.98 (2 H, d, J = 5.2 Hz), 7.05 ppm (2 H, d, J = 5.2 Hz). MS: See Figure 1.

Table II. Volatile Sulfur-Containing Components Identified in a Model Meat Flavor System

chem struct	no.	component name	occurrence in food or model system	Kovats index (DB-1)[a]
	1	2-methyl-3-furanthiol	Evers et al. (1976) Katz (1981) Golovnya et al. (1983a) Golovnya et al. (1983b) Hartman et al. (1984a) Hartman et al. (1984b) Hartman et al. (1984c) Reineccius and Liardon (1985) Ames and Mac Leod (1985)	846
	2	2-methyl-3-thiophenethiol	van den Ouweland and Peer (1975)	1030
	3	bis(2-methyl-3-furyl) disulfide	Evers et al. (1976) Katz (1981) Golovnya et al. (1983a) Golovnya et al. (1983b) Hartman et al. (1984a) Hartman et al. (1984b) Reineccius and Liardon (1985) Ames and Mac Leod (1985)	1494
	4	bis(2-methyl-3-thienyl) disulfide		1867
	5	2-methyl-3-[(2-methyl-3-thienyl)dithio]furan[b]		1681
	6	2,3-dihydro-5-methyl-4-[(2-methyl-3-furyl)dithio]furan[c]	Hartman et al. (1984c)	1627
	7	bis(2-methyl-4,5-dihydro-3-furyl) disulfide[c]	Hartman et al. (1984c)	1675

[a] 60 m × 0.32 mm (i.d.) DB-1; film thickness 0.25 μm; 60–220 °C at 3 °C/min. [b] Reported for the first time. [c] Tentatively identified.

Scheme I. Synthesis of 2-Methyl-3-[(2-methyl-3-thienyl)-dithio]furan (5)

2-methyl-3-furanthiol **1**   2-methyl-3-thiophenethiol **2**

2-methyl-3-(2-methyl-3-thienyldithio)-furan **5**
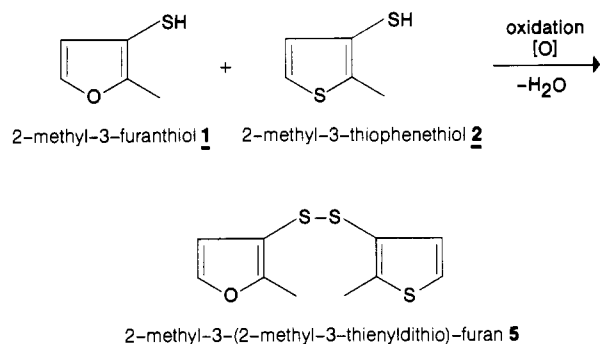
3. *Synthesis of 2-Methyl-3-[(cis-2-methyltetrahydro-3-thienyl)thio]furan (8) and 2-Methyl-3-[(trans-2-methyltetrahydro-3-thienyl)thio]furan (9)*. According to Durden and Weiden (1974), 3-mercaptopropionic acid readily reacts with 2-chloropropionic acid in 62% yield to give 2-methyl-3-thiaadipic acid. The acid from the preceding reaction was then heated to 250 °C with barium hydroxide and converted to 2-methylthiophan-3-one during pyrolysis in a yield of 80%. LiAlH₄ reduction of 2-methylthiophan-3-one gave a cis/trans mixture of the corresponding alcohol (2-methylthiophan-3-ol), which was allowed to react with p-tolylsulfonyl chloride with the procedure as described by van den Ouweland and Peer (1970).

Pure 2-methyl-3-[(p-tolylsulfonyl)oxy]tetrahydrothiophene was obtained in 90% yield.

The 2.72 g (10 mmol) of tosylate thus obtained was dissolved in ethanol. This solution was added drop by drop to a stirred mixture of 1.14 g (10 mmol) of 2-methyl-3-furanthiol and sodium methylate in ethanol in an atmosphere of nitrogen at ambient
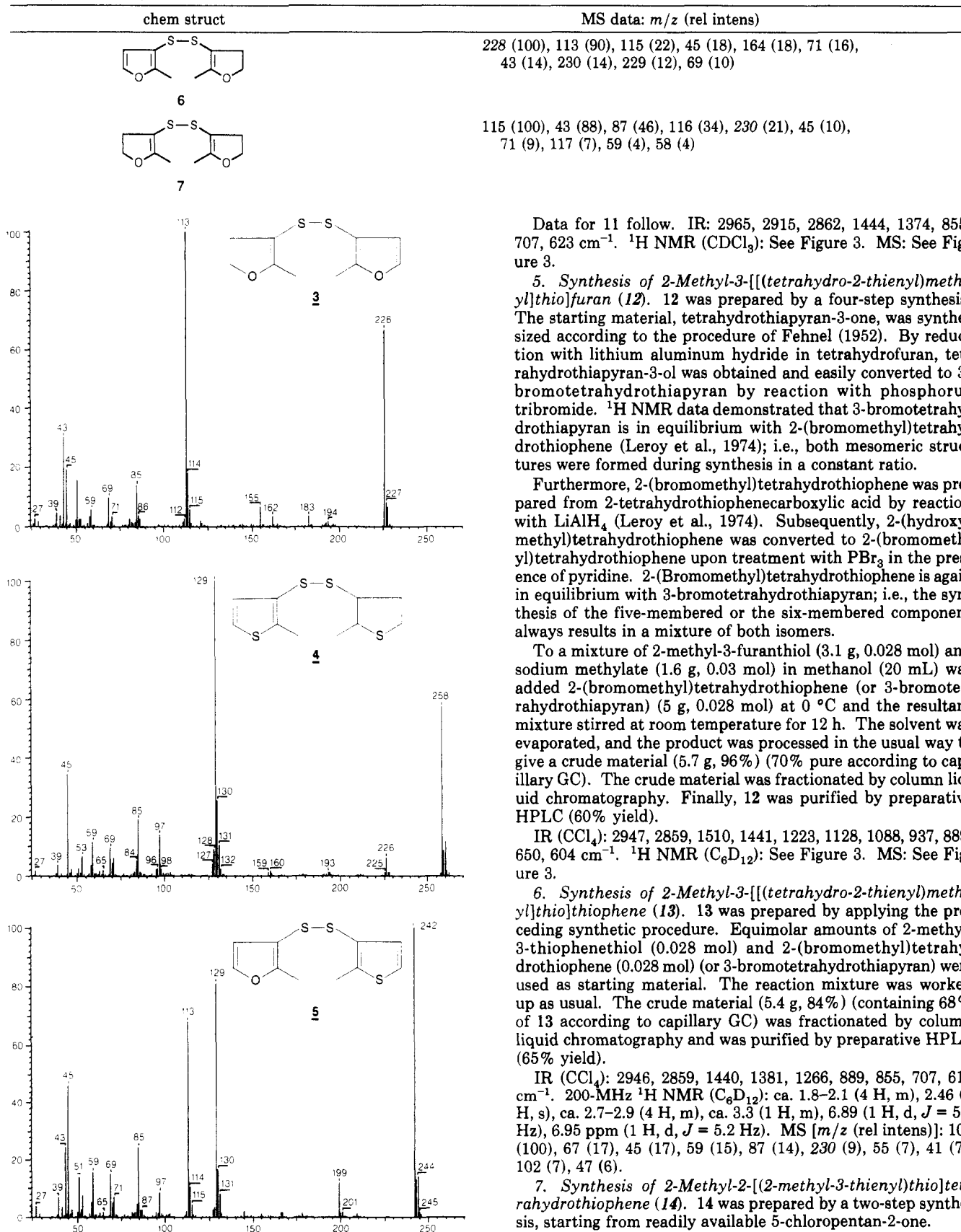
temperature. After completion of the addition, stirring at room temperature was continued for 24 h. Ethanol was evaporated and the residue quenched with water. Extraction with ether led to an oily product (0.98 g, 46%), which was further purified by liquid chromatography, and the pure isomers could be isolated by preparative capillary gas chromatography.

Data for 8 follow. IR: 2963, 2921, 2862, 1581, 1510, 1440, 1383, 1370, 1222, 1191, 1170, 1128, 1088, 940, 890, 652, 606 cm⁻¹. ¹H NMR (CDCl₃): 1.41 (3 H, d, J = 6.6 Hz), 1.84–2.04 (1 H, m), 2.36 (3 H, s), 2.28–2.43 (1 H, m), 2.76–2.94 (3 H, m), 3.09–3.24 (1 H, m), 6.32 (1 H, d, J = 2.0 Hz), 7.3 ppm (1 H, d, J = 2.0 Hz). MS [m/z (rel intens)]: 101 (100), 214 (53), 67 (40), 59 (37), 41 (18), 100 (17), 45 (16), 69 (14), 43 (12), 85 (11).

Data for 9 follow. IR: 2964, 2919, 2862, 1583, 1510, 1442, 1373, 1222, 1192, 1128, 1088, 1022, 937, 889, 652, 605 cm⁻¹. ¹H NMR (CDCl₃): 1.39 (3 H, d, J = 6.6 Hz), 1.94–2.28 (2 H, m), 2.35 (3 H, s), 2.84 (1 H, dt, J = 10.3 Hz, J = 7.2 Hz), 3.04 (1 H, ddd, J = 10.2 Hz, J = 7.7 Hz, J = 5.4 Hz), 3.38–3.56 (2 H, m), 6.35 (1 H, d, J = 1.9 Hz), 7.29 ppm (1 H, d, J = 1.9 Hz). MS [m/z (rel intens)]: 101 (100), 214 (54), 67 (36), 59 (35), 41 (16), 45 (15), 69 (11), 43 (11), 113 (10), 55 (9).

4. *Synthesis of cis-2-Methyl-3-[(2-methyl-3-thienyl)thio]-tetrahydrothiophene (10) and trans-2-Methyl-3-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (11)*. Both isomers 10 and 11 were prepared by applying the preceding synthetic procedure. Equimolar amounts of 2-methyl-3-[(p-tolylsulfonyl)oxy]tetrahydrothiophene (10 mmol) and 2-methyl-3-thiophenethiol (10 mmol) were allowed to react by the method described above. In order to prepare the corresponding sulfur compounds, the reaction mixture was worked up in the usual way (1.26 g, 55%) and the isomers were purified by column liquid chromatography as well as by preparative gas chromatography.

Data for 10 follow. IR: 2963, 2912, 2862, 1438, 1372, 855, 708, 622 cm⁻¹. ¹H NMR (CDCl₃): 1.4 (3 H, d, J = 6.7 Hz), 1.82–2.04 (1 H, m), 2.36 (1 H, m, J_1 = 10.4 Hz, J_2 = 5.0 Hz), 2.54 (3 H, s), 2.8–3.0 (3 H, m), 3.2 (1 H, m, J_1 = 6.5 Hz, J_2 = 8.5 Hz), 6.96 (1 H, d, J = 5.5 Hz), 7.09 ppm (1 H, d, J = 5.4

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Table III. Mass Spectral Data of 2,3-Dihydro-5-methyl-4-[(2-methyl-3-furyl)dithio]furan (6) and Bis(2-methyl-4,5-dihydro-3-furyl) Disulfide (7)

chem struct	MS data: m/z (rel intens)
6	*228* (100), 113 (90), 115 (22), 45 (18), 164 (18), 71 (16), 43 (14), 230 (14), 229 (12), 69 (10)
7	115 (100), 43 (88), 87 (46), 116 (34), *230* (21), 45 (10), 71 (9), 117 (7), 59 (4), 58 (4)

Figure 1. Mass spectra of bis(2-methyl-3-furyl) disulfide (3), bis(2-methyl-3-thienyl) disulfide (4), and 2-methyl-3-[(2-methyl-3-thienyl)dithio]furan (5).

Hz). MS [m/z (rel intens)]: 101 (100), *230* (46), 59 (37), 67 (34), 45 (24), 69 (20), 100 (19), 41 (18), 85 (14), 129 (10).

Data for 11 follow. IR: 2965, 2915, 2862, 1444, 1374, 855, 707, 623 cm^{-1}. ^{1}H NMR (CDCl$_3$): See Figure 3. MS: See Figure 3.

5. Synthesis of 2-Methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]furan (12). 12 was prepared by a four-step synthesis. The starting material, tetrahydrothiapyran-3-one, was synthesized according to the procedure of Fehnel (1952). By reduction with lithium aluminum hydride in tetrahydrofuran, tetrahydrothiapyran-3-ol was obtained and easily converted to 3-bromotetrahydrothiapyran by reaction with phosphorus tribromide. ^{1}H NMR data demonstrated that 3-bromotetrahydrothiapyran is in equilibrium with 2-(bromomethyl)tetrahydrothiophene (Leroy et al., 1974); i.e., both mesomeric structures were formed during synthesis in a constant ratio.

Furthermore, 2-(bromomethyl)tetrahydrothiophene was prepared from 2-tetrahydrothiophenecarboxylic acid by reaction with LiAlH$_4$ (Leroy et al., 1974). Subsequently, 2-(hydroxymethyl)tetrahydrothiophene was converted to 2-(bromomethyl)tetrahydrothiophene upon treatment with PBr$_3$ in the presence of pyridine. 2-(Bromomethyl)tetrahydrothiophene is again in equilibrium with 3-bromotetrahydrothiapyran; i.e., the synthesis of the five-membered or the six-membered component always results in a mixture of both isomers.

To a mixture of 2-methyl-3-furanthiol (3.1 g, 0.028 mol) and sodium methylate (1.6 g, 0.03 mol) in methanol (20 mL) was added 2-(bromomethyl)tetrahydrothiophene (or 3-bromotetrahydrothiapyran) (5 g, 0.028 mol) at 0 °C and the resultant mixture stirred at room temperature for 12 h. The solvent was evaporated, and the product was processed in the usual way to give a crude material (5.7 g, 96%) (70% pure according to capillary GC). The crude material was fractionated by column liquid chromatography. Finally, 12 was purified by preparative HPLC (60% yield).

IR (CCl$_4$): 2947, 2859, 1510, 1441, 1223, 1128, 1088, 937, 889, 650, 604 cm^{-1}. ^{1}H NMR (C$_6$D$_{12}$): See Figure 3. MS: See Figure 3.
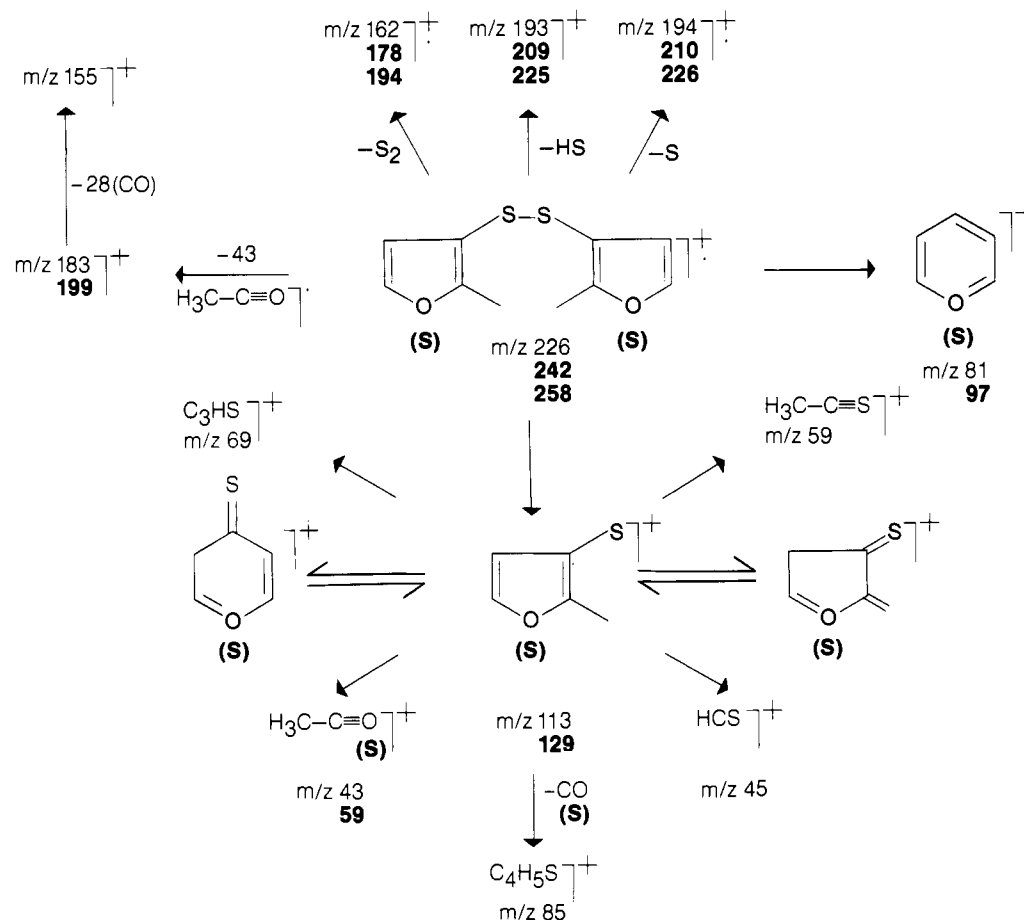
6. Synthesis of 2-Methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]thiophene (13). 13 was prepared by applying the preceding synthetic procedure. Equimolar amounts of 2-methyl-3-thiophenethiol (0.028 mol) and 2-(bromomethyl)tetrahydrothiophene (0.028 mol) (or 3-bromotetrahydrothiapyran) were used as starting material. The reaction mixture was worked up as usual. The crude material (5.4 g, 84%) (containing 68% of 13 according to capillary GC) was fractionated by column liquid chromatography and was purified by preparative HPLC (65% yield).

IR (CCl$_4$): 2946, 2859, 1440, 1381, 1266, 889, 855, 707, 618 cm^{-1}. 200-MHz ^{1}H NMR (C$_6$D$_{12}$): ca. 1.8–2.1 (4 H, m), 2.46 (3 H, s), ca. 2.7–2.9 (4 H, m), ca. 3.3 (1 H, m), 6.89 (1 H, d, J = 5.2 Hz), 6.95 ppm (1 H, d, J = 5.2 Hz). MS [m/z (rel intens)]: 101 (100), 67 (17), 45 (17), 59 (15), 87 (14), *230* (9), 55 (7), 41 (7), 102 (7), 47 (6).

7. Synthesis of 2-Methyl-2-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (14). 14 was prepared by a two-step synthesis, starting from readily available 5-chloropentan-2-one.

With a procedure analogous to that reported by Bateman and Glazebrook (1958), 40.8 g (340 mmol) of 5-chloropentan-2-one and 38.0 g (340 mmol) of potassium thioacetate were dissolved in 200 mL of ethanol. After the reaction mixture had remained at reflux temperature for 4 h, the precipitate formed was removed by filtration. To this solution was added water (150 mL). The mixture was extracted twice with 50 mL of diethyl

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Scheme II. Proposed Mass Spectral Fragmentation Steps of Heterocyclic Disulfides

m/z 162⌉⁺˙ m/z 193⌉⁺ m/z 194⌉⁺˙
178 **209** **210**
194 **225** **226**

m/z 155⌉⁺

−S₂ −HS −S

−28(CO)

m/z 183⌉⁺
199

−43
H₃C−C≡O⌉˙

(S) m/z 226 (S) (S)
242
258

m/z 81
97

C₃HS⌉⁺
m/z 69

H₃C−C≡S⌉⁺
m/z 59

(S) (S) (S)

H₃C−C≡O⌉⁺
(S)

m/z 113
129

HCS⌉⁺
m/z 45

m/z 43
59

−CO
(S)

C₄H₅S⌉⁺
m/z 85

ether. The combined extracts were dried with anhydrous sodium sulfate, evaporated to dryness, and finally distilled to give 28 g (53%) of 5-(acetylthio)pentan-2-one, bp 110–115 °C (13 mbar).

A mixture of 5-(acetylthio)pentan-2-one (2.4 g; 15 mmol) and sodium methylate (50 mg) in methanol (20 mL) was stirred for 18 h at ambient temperature, when 2-methyl-3-thiophenethiol (2.0 g, 15 mmol) in ethanol (20 mL) was added dropwise to the solution together with concentrated sulfuric acid (0.5 mL). After being heated at reflux for 5 h, the reaction mixture was neutralized with 1 M KOH (30 mL) and subsequently extracted twice with cyclohexane (30 mL). The combined organic layers were washed with water (30 mL), dried over anhydrous sodium sulfate, and evaporated to give a residue of 2.5 g (71.4%) (44% pure according to capillary GC), which was finally purified by preparative capillary GC.

IR (CCl₄): 2955, 2859, 1437, 1369, 1175, 1129, 1056, 854, 709, 655, 631 cm⁻¹. ¹H NMR (CDCl₃): See Figure 3. MS: See Figure 3.

Furthermore, 14 was prepared according to the procedure given in DOS 2458609 (de Roos et al., 1975) by reaction of 2-methyl-3-thiophenethiol and 2-methyl-4,5-dihydrothiophene.

8. Synthesis of 2-Methyl-3-[(2-methyltetrahydro-2-thienyl)thio]furan (15). 15 can be similarly synthesized from 5-(acetylthio)pentan-2-one (15 mmol) and 2-methyl-3-furanthiol (15 mmol) by the method described above. Only concentrated sulfuric acid was replaced by p-toluenesulfonic acid. Complete workup (1.98 g, 62%) (60% pure according to capillary GC), separation, and purification was as described for 14.

IR (CCl₄): 2951, 2919, 2858, 1512, 1438, 1222, 1125, 1087, 1056, 938, 888, 658, 606 cm⁻¹. ¹H NMR (CDCl₃): 1.70 (3 H, s), ca. 1.8 (1 H, m), ca. 2.1–2.58 ppm (3 H, m), 2.39 (3 H, s), ca. 3.04 (2 H, m), 6.40 (1 H, d, J = 1.9 Hz), 7.31 ppm (1 H, d, J = 1.9 Hz). MS [m/z (rel intens)]: 101 (100), 59 (19), 67 (14), 85 (11), 114 (10), 100 (9), 45 (8), 99 (7), 102 (7), 43 (6).

In addition, 15 was synthesized by reacting 2-methyl-3-furanthiol with 2-methyl-4,5-dihydrothiophene (de Roos et al.,

1975). Moreover, it is worth mentioning that it was not possible to purify component 14 by means of preparative liquid chromatography. With a pentane/ether silica gel chromatographic system, nearly total decomposition of 14 was observed.

9. Synthesis of (Methylthio)methanethiol (16) and 1-(Methylthio)ethanethiol (17). 16 was prepared according to the procedure given by Schutte (1971). Distillation of the crude product resulting from usual workup afforded 500 mg of (methylthio)methanethiol [bp 40 °C (11 Torr)], which was finally purified by preparative chromatographic techniques.
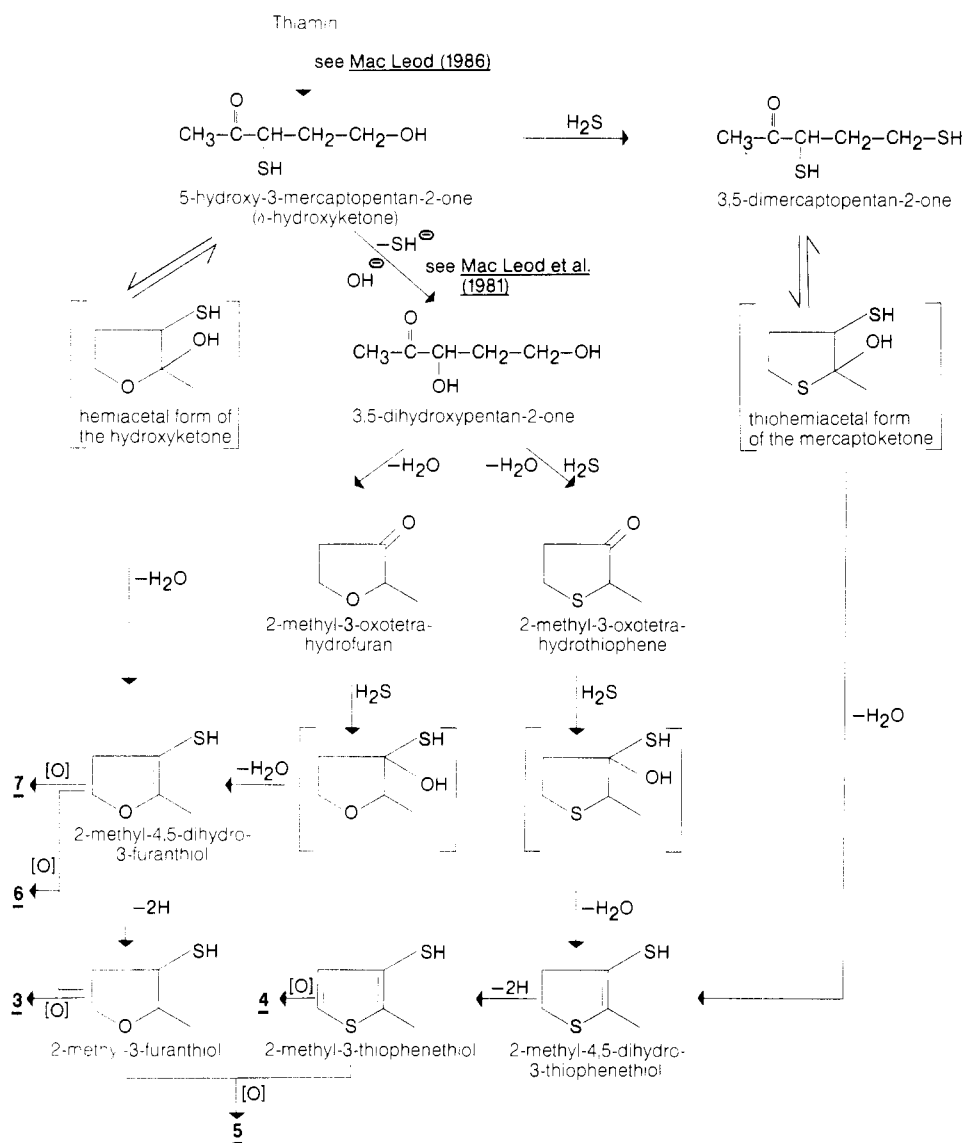
¹H NMR (CDCl₃): 1.89 (1 H, t, J = 8.0 Hz), 2.25 (3 H, s), 3.67 ppm (2 H, d, J = 8.0 Hz). MS [m/z (rel intens)]: 61 (100), 94 (84), 45 (75), 47 (65), 46 (44), 35 (26), 27 (22), 48 (17), 49 (8), 96 (8).

17 was synthesized from 1-(methylthio)-1-chloroethane [which can be prepared by the method described by Böhme and Bentler (1956)] and thiourea as previously described (Brinkmann and van der Heyden, 1971). Reaction of 1-(methylthio)-1-chloroethane with thiourea followed by alkaline treatment of the resulting thiouronium salt afforded 81% pure 1-(methylthio)ethanethiol [bp 66–74 °C (119 mbar)], which was further purified by chromatographic techniques.

IR (CCl₄): 2969, 2915, 2861, 1442, 1421, 1371, 1192, 1062, 953, 872, 698 cm⁻¹. ¹H NMR (CDCl₃): 1.68 (3 H, d, J = 6.9 Hz), 2.06 (1 H, d, J = 6.9 Hz), 2.24 (3 H, s), 4.03 ppm (1 H, quintet, J = 6.9 Hz). MS [m/z (rel intens)]: 75 (100), 61 (52), 108 (46), 59 (33), 45 (31), 47 (26), 60 (23), 41 (23), 27 (15), 58 (11).

10. Synthesis of 1-[(2-Methyl-3-thienyl)thio]ethanethiol (18). NaOH (4 g, 0.1 mol) was dissolved in water (36 mL) and the resultant mixture saturated with hydrogen sulfide at 0 °C. Methylene chloride (30 mL) and acetic acid (0.5 mL) were added. Afterwards acetaldehyde (4.4 g, 0.1 mol) along with 2-methyl-3-thiophenethiol (13 g, 0.1 mol) were added dropwise to the solution. The pH was adjusted to 5–6 by subsequent addition of acetic acid (ca. 10 g). Hydrogen sulfide was passed into the solution over 2 h at ambient temperature. This mixture was

Scheme III. Possible Formation Mechanisms for Heterocyclic Disulfides 3–7

Scheme showing formation mechanisms starting from Thiamin (see Mac Leod (1986)), through 5-hydroxy-3-mercaptopentan-2-one (α-hydroxyketone), reacting with H_2S to give 3,5-dimercaptopentan-2-one; hemiacetal form of the hydroxyketone; 3,5-dihydroxypentan-2-one (see Mac Leod et al. (1981)); thiohemiacetal form of the mercaptoketone; 2-methyl-3-oxotetrahydrofuran; 2-methyl-3-oxotetrahydrothiophene; 2-methyl-4,5-dihydro-3-furanthiol; 2-methyl-3-furanthiol (3); 2-methyl-3-thiophenethiol (4); 2-methyl-4,5-dihydro-3-thiophenethiol; leading to compounds 7, 6, 3, 4, and 5 via [O], −2H, −H₂O, and H₂S steps.

allowed to stand overnight at room temperature and was then alkalized with NaOH. The organic layer was separated and discarded. The aqueous solution was acidified with diluted hydrochloric acid, and the 1-[(2-methyl-3-thienyl)thio]ethanethiol released was recovered by extraction with diethyl ether. This ethereal extract was washed with one portion of water and then dried over anhydrous Na_2SO_4 after which the ether was expelled by distillation at ambient pressure. The crude product (3 g) was distilled at reduced pressure. Three fractions boiling at 71 °C (0.3 mbar) were collected. In this way, 0.3 g of the desired product was obtained in the form of a mobile clear liquid. 1-[(2-Methyl-3-thienyl)thio]ethanethiol was finally purified by preparative gas chromatography and characterized by ^1H NMR, IR, and mass spectra.

IR: 2969, 2920, 2859, 1443, 1371, 1193, 1178, 1091, 1050, 854, 710, 628 cm^{-1}. ^1H NMR (C_6D_{12}): 1.53 (3 H, d, J = 6.9 Hz), 1.99 (1 H, d, J = 6.5 Hz), 2.5 (3 H, s), 4.15 (1 H, quintet, J = 6.9 Hz), 6.95 (1 H, d, J = 5.3 Hz), 6.99 ppm (1 H, d, J = 5.3 Hz). MS [m/z (rel intens)]: 130 (100), 97 (29), 61 (26), 129 (25), 45 (22), 59 (17), 190 (16), 131 (11), 132 (9), 85 (8).

11. Synthesis of 1-[(2-Methyl-3-furyl)thio]ethanethiol (19). This component was prepared by applying the preceding synthetic procedure to 2-methyl-3-furanthiol. Distillation of the crude product (3.1 g) resulting from usual workup afforded 500 mg of 19, which was purified by preparative capillary GC.

IR (CCl_4): 2966, 2919, 2860, 1581, 1512, 1443, 1385, 1370, 1223,

1126, 1088, 1051, 939, 889, 654, 606 cm^{-1}. ^1H NMR (C_6D_{12}): See Figure 5. MS: See Figure 5.

RESULTS AND DISCUSSION

A similar meat flavor model system—monosodium glutamate, ascorbic acid, thiamin hydrochloride, and cystine—was investigated by another research group in 1984. Only 18 sulfur-containing flavor components could be identified during this earlier study (Hartman et al., 1984a,b). Obviously, previous work on volatile sulfur-containing components has not been very extensive, or the formation of sulfur-substituted flavor components proceeded quite differently due to different reaction conditions (e.g., effect of reaction temperature, reaction time, pH, solvent, or molar ratio). By way of contrast, a total of 70 sulfur-containing compounds were identified in the volatile components isolated from our model meat flavor system. A rough survey of the chemical classes represented in the processed meat aroma is shown in Table I. Of particular interest is the identification of five different types of heterocyclic sulfur-containing flavor components, the preponderance of which were furans and thiophenes substituted with sulfur in the 2- and 3-positions. The bulk of
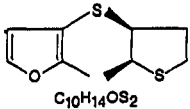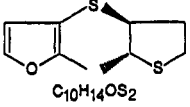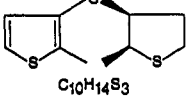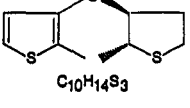
S-Containing Meat Flavor Components
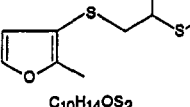
J. Agric. Food Chem., Vol. 38, No. 3, 1990 **783**
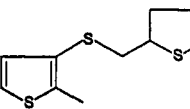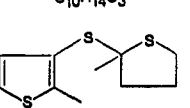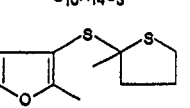
Chemical structure		Name of component	Kovats-Index DB-1
$C_{10}H_{14}OS_2$	8	2-methyl-3-(cis-2-methyl-3-tetrahydrothienylthio)-furan	1561
$C_{10}H_{14}OS_2$	9	2-methyl-3-(trans-2-methyl-3-tetrahydrothienylthio)-furan	1607
$C_{10}H_{14}S_3$	10	cis-2-methyl-3-(2-methyl-3-thienylthio)-tetrahydro-thiophene	1754
$C_{10}H_{14}S_3$	11	trans-2-methyl-3-(2-methyl-3-thienylthio)-tetrahydro-thiophene	1799
$C_{10}H_{14}OS_2$	12	2-methyl-3-(2-tetrahydro-thienylmethylthio)-furan	1645
$C_{10}H_{14}S_3$	13	2-methyl-3-(2-tetrahydro-thienylmethylthio)-thiophene	1845
$C_{10}H_{14}S_3$	14	2-methyl-2-(2-methyl-3-thienylthio)-tetrahydro-thiophene	1727
$C_{10}H_{14}OS_2$	15	2-methyl-3-(2-methyl-2-tetrahydrothienylthio)-furan	1537

Figure 2. Newly identified heterocyclic thioethers in a model meat flavor system.

these flavor compounds had not been identified in meat and had not been reported in the literature so far.

Identification of these components was based on GC/MS and retention index information. Novel compounds were isolated by preparative capillary gas chromatography and spectroscopically identified by interpretation of infrared, nuclear magnetic resonance, and mass spectra. In most cases, the structure was ultimately confirmed by chemical synthesis.

Furans and Thiophenes Substituted at the 3-Position with Sulfur. Table II lists some furan and thiophene components substituted with sulfur at the 3-position on both heterocyclic rings along with Kovats retention index data and references concerning the occurrence in foods or model systems.

The occurrence of 1 and 3 in a flavor model system has been pointed out by Hartman et al. (1984a,b). Moreover, 2-methyl-3-furanthiol (1) and bis(2-methyl-3-furyl) disulfide (3) have already been identified as major constituents in a model meat system that was prepared by refluxing an aqueous solution of cysteine hydrochloride, thiamin hydrochloride, and hydrolyzed vegetable protein for 4 h (Katz, 1981; Evers et al., 1976). Hartman et al. (1984c) as well as Reineccius and Liardon (1985) have recently studied the volatile products from thermally degraded thiamin. A number of different decomposition components were identified including 2-methyl-

Scheme IV. Synthesis of Cis/Trans Stereoisomers of 2-Methyl-3-[(2-methyltetrahydro-3-thienyl)thio]furan and 2-Methyl-3-[(2-methyl-3-thienyl)thio]tetrahydrothiophene

2-methyl-thiophan-3-one

1) LiAlH₄
2) p-TsCl

2-methyl-3-p-toluene-sulfonyloxy-tetrahydro-thiophene

(X=O,S)

(X=O $\longrightarrow$ **8, 9**/mixture of cis/trans stereoisomers)

(X=S $\longrightarrow$ **10, 11**/mixture of cis/trans stereoisomers)
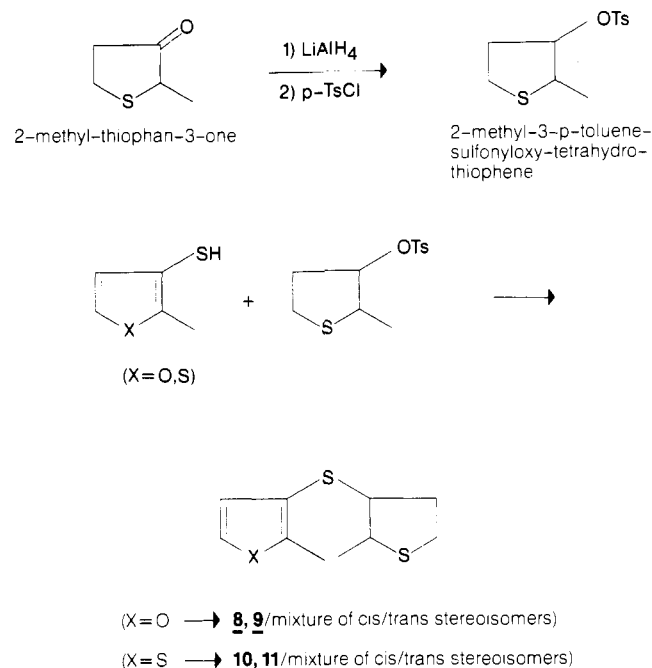
3-furanthiol and bis(2-methyl-3-furyl) disulfide. A schematic representation of the formation of these extremely important meat flavor compounds from thiamin is outlined by Mac Leod (1986). The odor threshold of bis(2-methyl-3-furyl) disulfide is remarkably low (Buttery et al., 1984). Similarly, comparably low odor thresholds are expected for the related structures shown in Table II.

Furthermore, 2-methyl-3-furanthiol and bis(2-methyl-3-furyl) disulfide were recently identified in the volatiles from a simulated meat flavor (Golovnya et al., 1983a,b) as well as from a heated yeast extract composition (Ames and Mac Leod, 1985).

2-Methyl-3-thiophenethiol (2) was identified in a heated model system of hydrogen sulfide with 4-hydroxy-5-methyl-3(2H)-furanone (norfuraneol) (van den Ouweland and Peer, 1975).

In contrast to 3, bis(2-methyl-3-thienyl) disulfide (4) is cited in the literature only once, in a Russian publication dealing with the synthesis and some transformations of sulfides of the thiophene series (Gol'dfarb et al., 1967). Bis(2-methyl-3-thienyl) disulfide was prepared from 2-methyl-3-thiophenethiol in our laboratory. This heterocyclic mercaptan was dimerized in an alkaline solution in the presence of H_2O_2. After the solvent was removed, the product was purified by fractional distillation. The synthetic compound proved to be identical with the flavor compound isolated from the complex model mixture. 2-Methyl-3-[(2-methyl-3-thienyl)dithio]furan (5) has not been previously described in the literature. This component was characterized as a main constituent in our model system. Furthermore, this component has also been identified in our laboratory in the headspace of beef quite recently (unpublished results). 5 was prepared by oxidizing a mixture of 2-methyl-3-furanthiol and 2-methyl-3-thiophenethiol. This is illustrated in Scheme I.

The structure assignment of reaction products 6 and 7 was solely based on the interpretation of MS and NMR data (microsamples) and was not confirmed by synthe-

Table IV. Sensory Properties of Heterocyclic Thioether Components 8–15

| name	odor quality description
2-methyl-3-[(cis-2-methyltetrahydro-3-thienyl)thio]furan (8)	herbaceous, nutty, carrot-like, terpene-like, mushroom-like, bread crust-like, meaty, grilled liver
2-methyl-3-[(trans-2-methyltetrahydro-3-thienyl)thio]furan (9)	roasted note, roasted filberts, carrot-like, terpene-like, potato-like, vegetable-like, asparagus-like, mushroom-like, meaty
cis-2-methyl-3-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (10)	carrot-like, terpene-like, mushroom-like, herbaceous, tropical fruit note, meaty, liver-sausage
trans-2-methyl-3-[(2-methyl-3-thienyl)thio]tetrahydro-thiophene (11)	roasted meat, burnt meat, meaty
2-methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]furan (12)	sulfury, leek-like, chives-like, garlic-like, estragole-like, oniony, slight meat character
2-methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]thiophene (13)	sulfury, rubbery, oniony, tropical fruit note, slight meat character
2-methyl-2-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (14)	metallic, oniony, cabbagy, burnt, typical meat note, characteristic roast meat aroma
2-methyl-3-[(2-methyltetrahydro-2-thienyl)thio]furan (15)	sulfury, metallic, minty, green, roasted, meaty, typical meat note
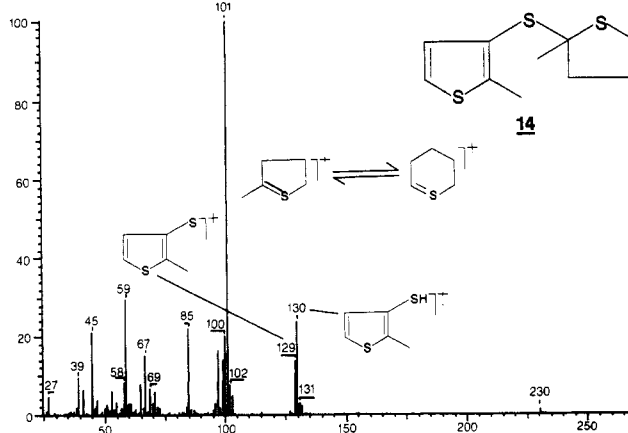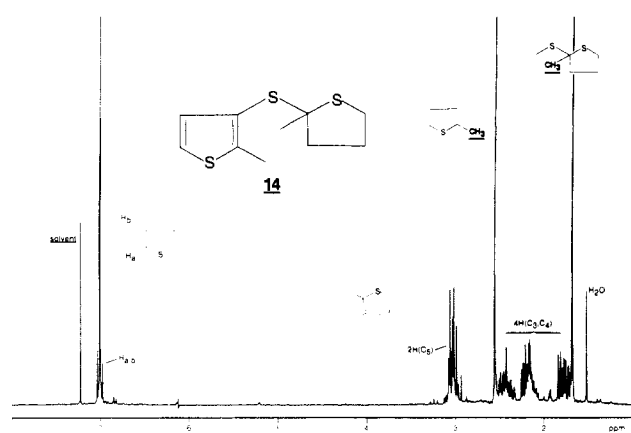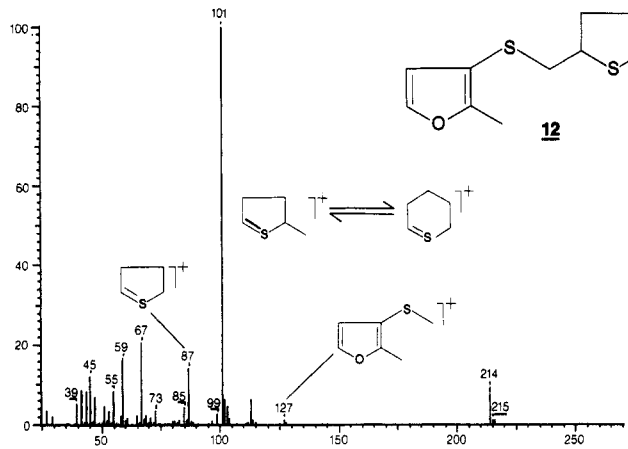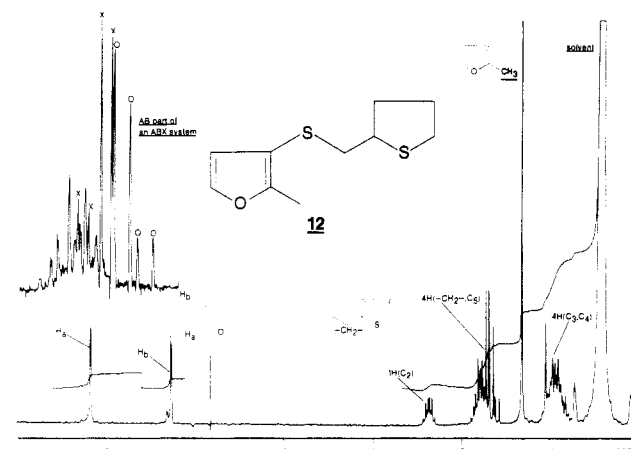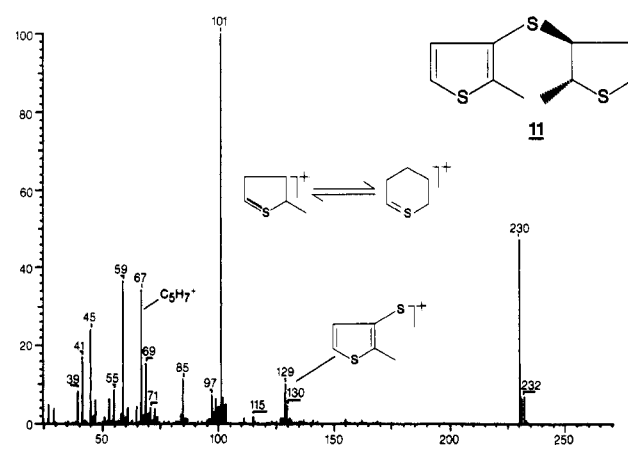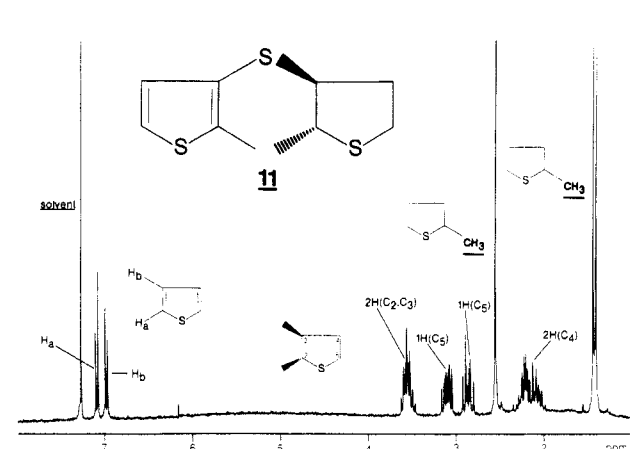
Figure 3. ^{1}H NMR and mass spectra of components 11, 12, and 14.

Table V. Spectroscopic Data of 2-Methyl-4,5-dihydrothiophene and 2-Methylenetetrahydrothiophene

chem struct	MS data: m/z (rel intens)	^{1}H NMR (CDCl$_3$): δ
	85 (100), 59 (78), *100* (73), 99 (62), 65 (30), 45 (28), 39 (24), 41 (12), 53 (10), 58 (9)	1.95 (3 H, m), 2.73 (2 H, m), 3.27 (2 H, t, *J* = 8.6 Hz), 5.26 (3 H, m)
	100 (100), 58 (50), 99 (28), 67 (24), 39 (23), 71 (19), 59 (18), 85 (18), 60 (17), 45 (16)	

Scheme V. Possible Formation Pathways to Heterocyclic Thioether Components 8 and 9

8 and **9**
(mixture of cis/trans stereoisomers)
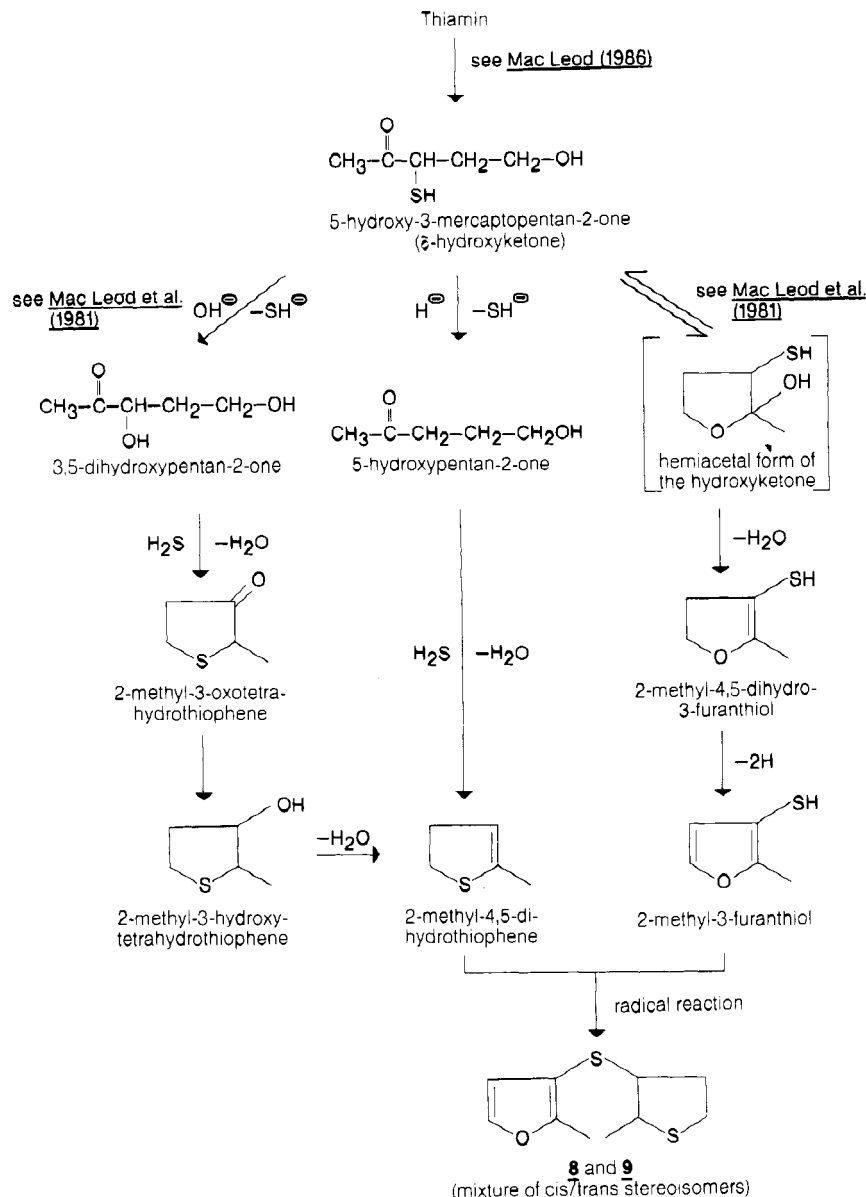
sis. Therefore, 6 and 7 are only considered as tentatively identified. Mass spectral fragmentations (major MS fragments) are listed in Table III. Both components have already been mentioned in the literature from the thermal degradation of thiamin (Hartman et al., 1984c). However, it is worth mentioning that our spectroscopic data for 6 and 7 are not in accordance with MS data published by Hartman et al. (1984c).

The mass spectra of the heterocyclic disulfides 3–5 appear in Figure 1. Proposed mass spectral fragmentation steps of heterocyclic disulfides are summarized in Scheme II. Generally, it should be noted that all sulfur structures described in the present paper showed a molecular ion in their mass spectra. In contrast to the electron impact mass spectra of oxygen components, the molec-

ular ions of sulfur substances are very often easily recognized due to the higher ionization potential of sulfur-containing compounds. Equally, sulfur-containing fragment ions are significantly more stable compared to the corresponding oxygen-containing fragment ions. For example, the stability of the thiapyrilium cation (m/z 97) is higher than that of the pyrilium cation (m/z 81).

All three mass spectra of the heterocyclic disulfides are characterized by intensive molecular ions. The main feature of the fragmentation is the cleavage of the disulfide bond system, giving intensive fragments at m/z 113 ((2-methyl-3-furyl)thio cation) and m/z 129 ((2-methyl-3-thienyl)thio cation). The presumed structures of these fragments formed by intramolecular rearrangements are also presented in Scheme II. It is worthwhile to men-

Scheme VI. Synthesis of 2-Methyl-3-[[(tetrahydro-2-thi-enyl)methyl]thio]furan (12) and 2-Methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]thiophene (13)
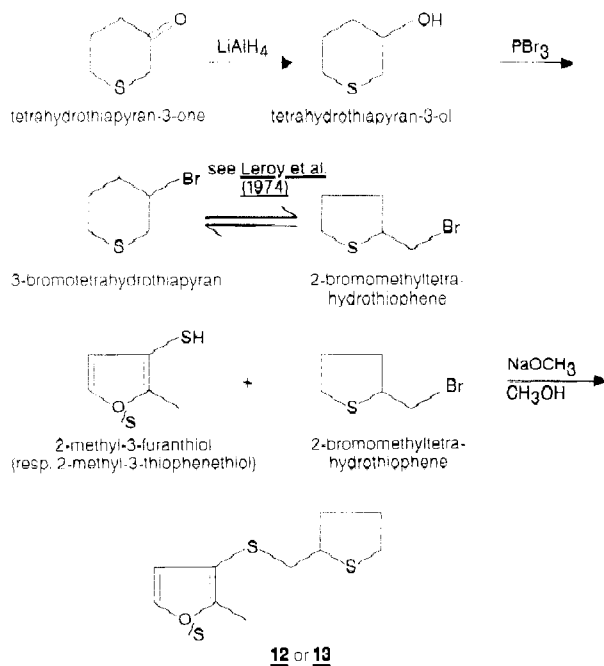
tion that the fragment ion m/z 43 (acetyl cation) plays an important role in the fragmentation process of these disulfides and thioether compounds. This ion is a clue to the presence of the (2-methyl-3-furyl)thio structural fragment for this type of sulfur-containing component. Accordingly, m/z 43 is not present in the mass spectrum of 4 due to the absence of the (2-methyl-3-furyl)thio part. The ions m/z 183 and 199 arise from the loss of an acetyl radical from the molecular ions of 3 and 5. The peak at m/z 155 in the mass spectrum of 3 probably results from m/z 183 by loss of carbon monoxide, thus indicating the second (2-methyl-3-furyl)thio part in this structural formula.

1 and 3 possess characteristic meat flavor notes and are likely to be of prime importance in cooked meat aromas (Mac Leod, 1986). 2 exhibits a strong odor and flavor of roasted meat while 4 has less powerful organoleptic properties and is described as sulfurous, metallic, and rubbery, having only a slight meat character. Compound 5 delivers a meaty aroma but has additionally an allium-like flavor of onion or garlic with metallic and fatty background notes. The presence of the heterocyclic disulfides in the aroma mixture is easy to understand and can be generally postulated as oxidative decomposition products of the corresponding monomers. Even air oxidation of the monomers may result in dimerization without effort. The formation of component 5 in our model system, for example, is likely to occur by the same route outlined in Scheme I. Possible mechanisms for the formation of further heterocyclic disulfides are summarized in Scheme III.

Heterocyclic Thioethers. Another important type of sulfur compound identified in the model reaction mixture is represented in Figure 2. Eight heterocyclic thioethers were identified for the first time. Both heterocyclic ring systems of components 8–11 are substituted by sulfur in the 3-position. Due to the connection of a furan or thiophene ring system with a substituted tetrahydrothiophene structure, the formation of cis and trans stereoisomers is possible. This class of flavor com-

ponents has not previously been reported in a food system or in a model system. To confirm these structures, we have prepared compounds 8–11 by synthesis from 2-methyl-3-furanthiol or 2-methyl-3-thiophenethiol and 2-methyl-3-[(p-tolylsulfonyl)oxy]tetrahydrothiophene (Scheme IV). IR, ¹H NMR, and MS data of components 8–10 are summarized in the Experimental Section. The ¹H NMR and the mass spectrum of component 11 are presented in Figure 3.

The intensive ions at m/z 214 and 230 in the mass spectra of 8–11 indicate the molecular weights. In all spectra m/z 101 represents the base peak, which is presumably due to a 2-methyldihydrothienyl or dihydrothiopyranyl cation. Furthermore, the fragment ion at m/z 67 is rather characteristic for this type of heterocyclic thioether components. In all probability, m/z 67 refers to $C_5H_7{}^+$, which is formed from m/z 101 by loss of hydrogen sulfide. It is remarkable, however, that component 9 shows no visible elimination of m/z 43 though a (2-methyl-3-furyl)thio element is included in the structural formula. Equally, no loss of a thioacetyl radical could be observed from thioether components containing a (2-methyl-3-thienyl)thio element in their structural formula. The mass spectral fragmentation patterns of 8 and 10 are almost completely identical with those of 9 and 11, with the interesting exception of isomers 8 (cis) and 10 (cis) displaying a significantly higher ion at m/z 100 (about 18%) than isomers 9 (trans) and 11 (trans) (about 5%).

¹H NMR spectra of 8 and 9 are very similar just as the ¹H NMR spectra of 10 and 11. The only difference between both stereoisomers consists of the chemical shift values of the tetrahydrothiophene ring protons. The cis/trans stereochemistry was established by referring to the 2-methyl-3-thiolanol isomers (Zabransky et al., 1976) as well as to the 2-methyl-3-mercaptotetrahydrothiophene stereoisomers, which were assigned by van den Ouweland and Peer (1970) on the basis of retention times and spectroscopic data.

Furthermore, the ¹H NMR spectrum of 8 is also very close to the nuclear magnetic resonance spectrum of 10 while the ¹H NMR spectrum of 9 is very close to that of 11. The main difference is due to the aromatic protons of the furan and thiophene ring system.

Scheme V shows a possible mechanism for the formation of the two novel heterocyclic thioether components 8 and 9.

Completely analogous to this formation pathway, a possible reaction scheme is conceivable for the formation of the heterocyclic thioethers 10 and 11 via a radical mechanism from 2-methyl-4,5-dihydrothiophene and 2-methyl-3-thiophenethiol (formed via 2-methyl-3-oxotetrahydrothiophene; see Scheme III).

Components 8–11 are described as being strongly odorous. These components also illustrate the fact that, in spite of similarities in chemical structure, there are differences in sensory quality. The qualitative odor and/or flavor descriptions are compiled in Table IV. The flavor thresholds in water of 8 and 9 are below 1 ppb, and the flavor thresholds in water of 10 and 11 are below 100 ppb.

Two new substances, 12 and 13, which had not been previously identified as flavor compounds, are also depicted in Figure 2. These components are also heterocyclic thioethers and have the molecular formulas $C_{10}H_{14}OS_2$ and $C_{10}H_{14}S_3$, respectively.

Compound 12 was identified as 2-methyl-3-[[(tetrahydro-

Scheme VII. Possible Mechanisms for the Formation of 2-Methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]furan (12) and 2-Methyl-3-[[(tetrahydro-2-thienyl)methyl]thio]thiophene (13)
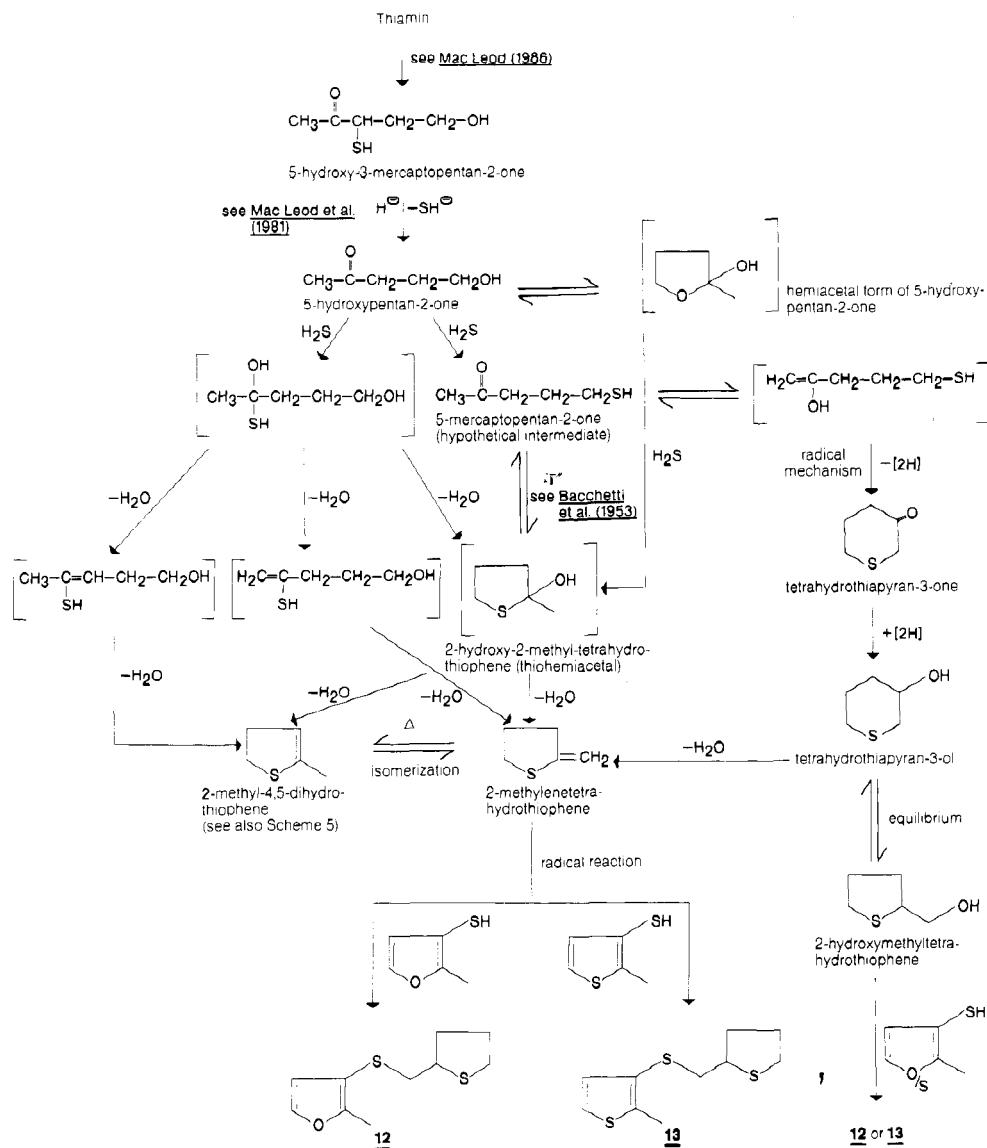
2-thienyl)methyl]thio]furan, and compound 13 was identified as 2-methyl-3-[[(tetrahydro-2-thienyl)methyl]-thio]thiophene. Accordingly, the furan or thiophene ring system is substituted with sulfur in the 3-position while the tetrahydrothiophene ring is connected via the 2-position. Thus, a characteristic feature of these components is the thiomethylene bridge between the two heterocyclic ring systems. The definite structural proof of these two thioether compounds was given again by synthesis as shown in Scheme VI.

Figure 3 exhibits the mass spectrum of 12. Base peak again is the ion at m/z 101. The abundance of the molecular ion is very low. A very important fragment ion is m/z 87, which is significantly present in the mass spectrum of 12. Obviously, m/z 87 stands for a dihydrothienyl cation and is therefore an essential indicator for the presence of a thiomethylene group between the two ring systems just as the fragment ions at m/z 127 or 143, which are also only present in conjunction with structure 12 or 13.

The ^{1}H NMR spectrum of 12 is displayed in Figure 3. The most striking feature of the ^{1}H NMR spectrum of 12 is the AB part of an ABX system. It could be assigned to the thiomethylene unit. Structure 12 is furthermore

ascertained by the absence of an aliphatic methyl group contrary to the ^{1}H NMR spectra of 8 and 10.

The ^{1}H NMR spectrum of 13 shows the well-known signals of a thiophene derivative. All other signals were similar to the spectrum of 12. Though the ABX system was not so evident as in the spectrum of 12, there was no doubt about the analogous structure.

Scheme VII shows two possible radical reaction pathways that could take place to generate 12 and 13 in our model system. Sensory properties of 12 and 13 are summarized in Table IV.

In Figure 2 two additional heterocyclic thioethers are shown that have not been identified as natural products thus far. Their structures were elucidated on the bases of infrared, ^{1}H NMR, and mass spectrometry to be 2-methyl-2-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (14) and 2-methyl-3-[(2-methyltetrahydro-2-thienyl)thio]furan (15); i.e., in this case the thioethers are substituted with sulfur in the furan or thiophene ring systems at the β-position while the sulfur in the tetrahydro part of the structure is at the α-position. The mass spectrum of 14 is presented in Figure 3 as well as the ^{1}H NMR data. The mass spectra of 14 and 15 are characterized by only minor parent ions at m/z 214 and 230, respectively. Again,

Scheme VIII. Synthesis of 2-Methyl-2-[(2-methyl-3-thienyl)thio]tetrahydrothiophene (14) and 2-Methyl-3-[(2-methyltetrahydro-2-thienyl)thio]furan (15)

Scheme IX. Formation Mechanisms Proposed for 14 and 15
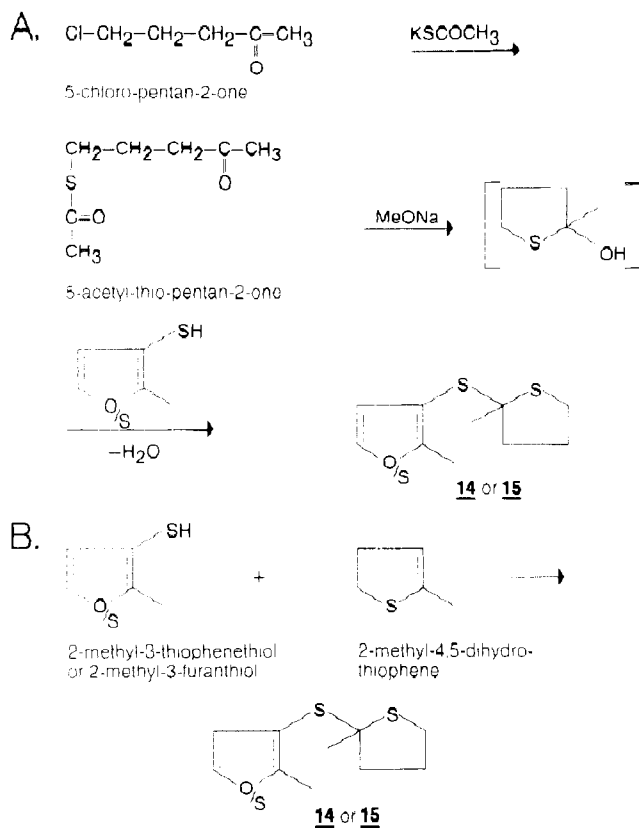
the base peak is due to the 2-methyldihydrothienyl cation at m/z 101, which loses a H_2S molecule to give the characteristic fragment ion at m/z 67. Contrary to the mass spectra of mercaptans or disulfides, however, the mass spectra of various thioether compounds (e.g., 8, 12, and 15) show no loss of an acetyl cation (m/z 43) or the thioacetyl cation (m/z 59), respectively. Summing up, it may be said that the mass spectra of the isomeric heterocyclic thioether structures are very similar and mainly differ in the stability (i.e., the relative intensity) of the molecular and few specific ions.

Primarily, the structures of 14 and 15 are confirmed by their ^1H NMR data. The detailed ^1H NMR data of component 15 are included in the Experimental Section while the ^1H NMR spectrum of structure 14 is shown in Figure 3. The methyl singlet at 1.7 ppm and the presence of only one methylene group adjacent to the sulfur atom lead to the 2,2-disubstituted tetrahydrothiophene structure of component 14.

For the sake of completeness, it must be noted that both components have already been described in the patent literature for use in gravies and meat products (de Roos et al., 1975).

The routes by which 14 and 15 were synthesized are shown in Scheme VIII. A probable pathway for the formation of 14 and 15 is outlined in Scheme IX. The organoleptic impressions of 14 and 15 are described in Table IV.

As far as the sensory evaluation of the various types of heterocyclic thioethers is concerned, it is worth noting that most of the heterocyclic thioethers are highly potent flavor components and are mainly associated with a meatlike odor impression at low concentration. At higher concentration, however, they show a terpene-like and/or
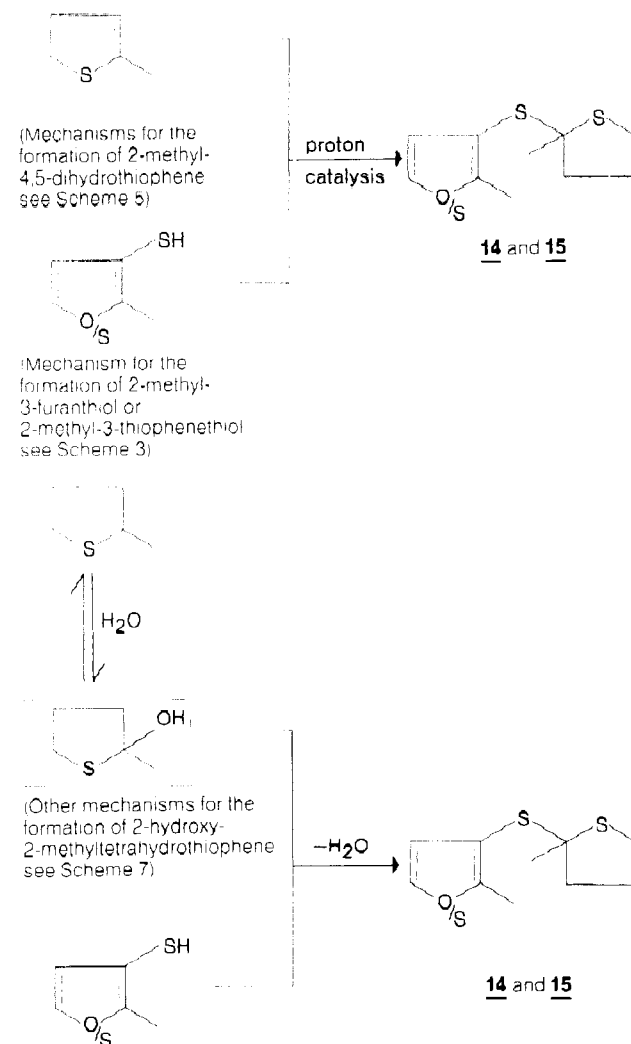
Chemical structure		Name of component	Kovats-Index DB-1
	16	methylthiomethanethiol	793
	17	1-methylthioethanethiol	815
	18	1-(2-methyl-3-thienylthio)-ethanethiol	1396
	19	1-(2-methyl-3-furylthio)-ethanethiol	1214

Figure 4. Newly identified hemidithioacetals in a model meat flavor system.

carrot-like character, while an intense sulfur odor is observed in a pure state. Generalizing from these observations, it may be said that the odor quality of the newly identified thioether components highly depends on the substance concentration and may change from one con-
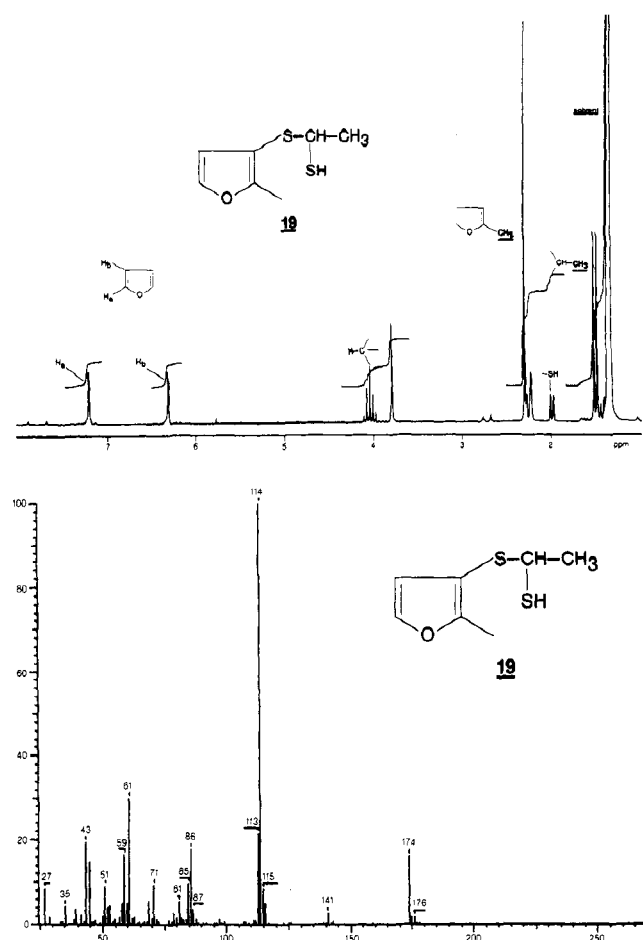
Figure 5. ¹H NMR and mass spectrum of component 19.

Scheme X. Synthesis of
1-[(2-Methyl-3-thienyl)thio]ethanethiol (18) and
**1-[(2-Methyl-3-furyl)thio]ethanethiol (19)**

centration to another. Many compounds are meaty only at certain concentrations, usually very low concentrations.

Aliphatic and Heterocyclic Hemidithioacetals. Finally, we report on four additional important flavor compounds that we have isolated and identified from our model mixture, two of which are, to the best of our knowledge, new to the literature. The components are illustrated in Figure 4. Two aliphatic hemidithioacetals, (methylthio)methanethiol (16) and 1-(methylthio)ethanethiol (17), as well as two heterocyclic hemidithioacetals, 1-[(2-methyl-3-thienyl)thio]ethanethiol (18) and 1-[(2-methyl-3-furyl)thio]ethanethiol (19), were identified.

The synthesis of (methylthio)methanethiol has already been described in the literature (Moir et al., 1980; Ohsaku et al., 1972; Fehér and Vogelbruch, 1958; Weissflog and Schmidt, 1979), but the component has never been mentioned in the context of flavor chemistry. Thus, (methylthio)methanethiol is reported here for the first time as flavor component. In contrast with 16, 1-(methylthio)-ethanethiol was reported in the headspace volatiles of beef broth by Brinkmann et al. (1972) and was described as having the odor of fresh onions. This component is formed when ethanal, methanethiol, and H₂S are heated in aqueous solution at pH 6 (Schutte and Koenders, 1972).

The chemical class of the thiaalkanethiols has been reported by different authors as flavor constituents (Golovnya et al., 1983a,c; Boelens et al., 1974; Golovnya and Rothe, 1980; Bodrero et al., 1981). According to the patent literature, 1-(methylthio)ethanethiol is extremely useful for meat flavors (Brinkmann and van der Heyden, 1971, 1972). A one-step synthesis of hemidithioacetals was published by Schutte (1971). Spectroscopic data
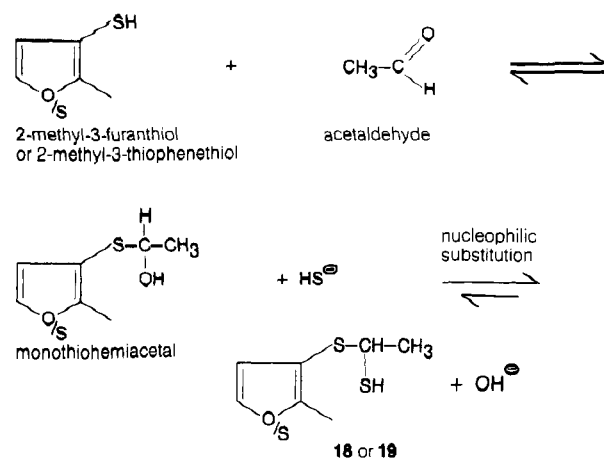
for 16 and 17 are summarized in the Experimental Section.

The other two heterocyclic hemidithioacetals shown in Figure 4 are, to our knowledge, reported here for the first time.

18 possesses sulfurous, carrot-like, leek-like, but also meaty flavor notes. This component imparts pleasant and interesting meaty, yeast-like, and onion-like flavors to food products. 18 improves the taste and/or smell of meat products by giving them a boiled meat flavor as well as a typical brothy character.

Organoleptic properties of 19 were reported by several members of the panel. For example, terms like roasted, brothy, spicy, onion, garlic, vegetable, meat, and gravy were frequently used. In particular, 19 has a powerful flavor with good meat character in a highly dilute solution. It possesses a typical savory meat note reminiscent of roast beef. Both components possess more interesting olfactory properties than the thioether substances. Therefore, it is likely that these components are mainly responsible for the interesting sensory properties of the model meat flavor system. Their flavor thresholds in water are below 50 ppt.

In Figure 5 the mass and ¹H NMR spectra of 19 are presented. The structures suggested for 18 and 19 were ultimately confirmed by synthesis.

18 and 19 were synthesized from hydrogen sulfide, acetaldehyde, and 2-methyl-3-thiophenethiol (or 2-methyl-3-furanthiol) and compared directly with the hemidithioacetals isolated by preparative capillary gas chromatography from the model system. The synthetic procedure is shown in Scheme X. Both synthetic components, 18 and 19, proved to be identical with their "natural" counterparts. We believe that these components form in the model system in a manner similar to that by which they are synthesized, because acetaldehyde and hydrogen sulfide are the primary thermal degradation products from cystine.

Furthermore, 1-[(2-methyl-3-furyl)thio]propanethiol could be identified tentatively based on mass spectral and retention index data. The mass spectrum looks significantly like a homologue of 19. The structure was assigned neither by ¹H NMR data nor by chemical synthesis.

In summary, model studies are very efficient for the identification and structure elucidation of important flavor components. Most of the compounds reported here

have not been identified in meat and have not yet been reported as constituents of food volatiles. Nevertheless, there are good reasons to believe that minute traces of these sulfur-containing components are present in roasted and/or cooked meat volatiles because our model system was based solely on naturally occurring precursors. We assume that only minute trace amounts of these types of components need to be present in natural products to be of prime significance due to their extremely low odor threshold values.

Finally, it should be noted that the mechanisms proposed for the formation of all heterocyclic thioethers are substantiated by the fact that we were able to isolate and identify 2-methyl-4,5-dihydrothiophene in our model meat flavor system. This component, which holds a central position in most proposed formation pathways (see Schemes V, VII, and IX), is present in our model mixture in a considerably high concentration. This is easy to understand, since 2-methyl-4,5-dihydrothiophene is the most predominant compound found from the thermal degradation of thiamin (Maga, 1975). In addition, the formation of 2-methyl-4,5-dihydrothiophene may follow various other pathways as can be seen in Scheme V and VII.

Likewise, 2-methylenetetrahydrothiophene could also be identified in our model meat flavor system, thus supporting the proposed formation mechanisms for components 12 and 13 (see Scheme VII).

The spectroscopic data of 2-methyl-4,5-dihydrothiophene and 2-methylenetetrahydrothiophene are shown in Table V.

To our surprise, we were not able to detect just one heterocyclic sulfur component comprised of a tetrahydrofuran moiety though the formation of such compounds should be quite possible due to the formation of 2-methyl-4,5-dihydrofuran, which has been identified by several research groups as a thermally degraded thiamin product. We have synthesized some tetrahydrofuran components in the hope that chromatographic and spectroscopic data will assist us in characterization of these tetrahydrofuran structures in our model system. All experiments in this direction, however, failed. A possible explanation of this phenomenon may be the fact that we were not able to identify 2-methyl-4,5-dihydrofuran in our reaction mixture. Probably, this component is present only in such a small amount—if at all—that it cannot play an important role as precursor for heterocyclic thioethers or disulfides containing a tetrahydrofuran structural element.

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